**Comments on the Weight of Evidence Cancer Conclusions in the Trichloroethylene: Consideration of Both Toxicological and Epidemiologic Evidence - External Review Draft**

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## Summary

These comments address the question of whether the overall toxicological and epidemiologic data provide sufficient evidence for description of TCE as "Carcinogenic to Humans." First we review the Environmental Protection Agency's (EPA's) 2005 guidelines for weight of evidence descriptors regarding carcinogenic potential . We then consider where the scientific evidence from toxicological and epidemiologic research best fits under these criteria.

Our key overall observations and conclusions are as follows: EPA has proposed a cancer descriptor of "carcinogenic to humans" for TCE "based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer."

Upon a critical scientific assessment, we find that the currently available are clearly not convincing of a causal association between TCE exposure and cancer in humans. This is because neither the epidemiologic data nor the animal and mechanistic data meet EPA's criteria of "carcinogenic to humans" as described in the 2005 EPA Guidelines for Carcinogen Risk Assessment. Moreover, we find that EPA has not judged any other chemical as a "human carcinogen" or its equivalent (using older guidelines) on such inconsistent support and such a lack of strong and convincing epidemiologic evidence. EPA's proposal to use the classification

"carcinogenic to humans" for TCE would be a poorly supported precedent in the application of its own guidelines.

Rather, our judgment based on the 2005 EPA Guidelines for Carcinogen Risk Assessment, which EPA has established to make such determinations consistent across chemical assessments, indicates that a more correct classification for EPA to make for TCE would either be "likely to be carcinogenic to humans" or "suggestive evidence of carcinogenicity" depending on how one considers the "adequacy" of evidence to demonstrate carcinogenic potential.

**Summary of EPA Guidelines**

The EPA's (2005) Guidelines for Carcinogen Risk Assessment suggest the following descriptors as an introduction to the weight of evidence (WOE) narrative, noting that the entire narrative provides the conclusions and the basis for them:

- Carcinogenic to humans,
- Likely to be carcinogenic to humans,
- Suggestive evidence of carcinogenicity,
- Inadequate information to assess carcinogenic potential, and
- Not likely to be carcinogenic to humans.

According to the guidelines, the descriptor "**carcinogenic to humans**" "indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- "This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

- Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* [italics added] of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action (MOA) but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on MOA and also an indication of the relative weight that each source of information carries, e.g., based on human information, based on limited human and extensive animal experiments."

According to the guidelines, the descriptor "**likely to be carcinogenic to humans**" is "appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor 'Carcinogenic to Humans.' Adequate evidence consistent with this descriptor covers a broad spectrum.... Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer;
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site or exposure route, with or without evidence of carcinogenicity in humans;

- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy or an early age at onset;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence."

According to the guidelines, the descriptor "**suggestive evidence of carcinogenicity**" is "appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples [of supporting data for this descriptor] include:

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans;"
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;

- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence; or
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

According to the guidelines, the descriptor "**inadequate information to assess carcinogenic potential**" is "appropriate when available data are judged inadequate for applying one of the other descriptors. Additional studies generally would be expected to provide further insights. Some examples include:

- little or no pertinent information;
- conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative;
- negative results that are not sufficiently robust for the descriptor, "not likely to be carcinogenic to humans."

**Application of the Guidelines to Trichloroethylene**

In considering the data in the context of applying the "carcinogenic to humans" descriptor, one first considers the weight of the epidemiological evidence. We judge the epidemiologic evidence to be neither "convincing" nor "strong," two key terms in the guidelines. This judgment is based on four recent reviews and meta-analyses of occupational TCE exposures and cancer as well as other reviews of this literature (Alexander et al., 2006, 2007; Mandel et al., 2006; Kelsh et al., 2010). The recent review and meta-analysis by Kelsh et al., 2010 focuses on occupational TCE exposure and kidney cancer, and includes the recent Charbotel 2006 study that is emphasized in the EPA assessment and used by EPA scientists to conduct a quantitative risk

assessment. Both the EPA meta-analysis and the recently published Kelsh et al. meta-analysis of the TCE-kidney cancer epidemiologic literature produced similar summary results. However in Kelsh et al., the limitations of this body of research, namely exposure assessment limitations, potential unmeasured confounding, potential selection biases, and inconsistent findings across groups of studies, did not allow for a conclusion that there is sufficient evidence of a casual association, despite a modest overall association. In addition, although the recent Charbotel et al. 2006 study has made important improvements in exposure assessment, it still has important potential limitations that do not permit an appropriate use in quantitative risk assessment.

There are reasonably well designed and well conducted epidemiologic studies that report no association between TCE and cancer, some reasonably well designed and conducted studies that did report associations between TCE and cancer, and finally some relatively poorly designed studies reporting both positive and negative findings. Overall, the summary relative risks or odds ratios in the meta-analysis studies (EPA or published meta-analyses) generally ranged between 1.2 and 1.4. The IRIS document refers to these associations as "small," a term not typically consistent with "convincing" and strong." Weak or small associations may be more likely to be influenced or be the result of confounding or bias. Smoking and body mass index are well-established risk factors for kidney cancer, and smoking and alcohol are risk factors for liver cancer, yet the potential impact of these factors on the meta-analysis associations was not fully considered. There were suggestions that these factors may have impacted findings (e.g. in the large Danish cohort study of TCE exposed workers, the researchers noted that smoking was more prevalent among the TCE exposed populations however little empirical data were provided (Raachou-Nielson et al., 2003). In addition, colinearity of occupational exposures (i.e., TCE exposure correlated with chemical and/or other exposures) may make it difficult to isolate

potential effects of TCE from those of other exposures within a given study, and hinder interpretation across studies. For example, although Charbotel et al. (2006) reported potential exposure response trends, while controlling for many confounders of concern (which strengthens the weight of evidence), they also reported attenuated associations for cumulative TCE exposure after adjustment for exposure to cutting fluids and other petroleum oils (weakening the weight of the evidence). This study is also be limited due to other by potential study design considerations such as selection bias, self report of work histories, residual confounding and other design factors.

When examining the data for TCE and non-Hodgkin lymphoma, kidney cancer, and liver cancer, associations were inconsistent across occupational groups (summary results differed between aerospace/aircraft worker cohorts compared with workers from other industries), study design, location of the study, quality of exposure assessment (e.g., evaluating studies that relied upon biomonitoring to estimate exposure vs. semi-quantitative estimates vs. self-report, etc.), and by incidence vs. mortality endpoints. Although EPA examined high dose categories, it did not evaluate any potential dose-response relationships across the epidemiologic studies (except for the Charbotel et al. 2006 study). In our reviews of the epidemiologic data reported in various studies for different exposure levels (e.g. cumulative exposure and duration of exposure metrics), we did not find consistent dose-response associations between TCE and the three cancer sites under review (Mandel et al., 2006; Alexander et al., 2007; Kelsh et al., 2010) An established dose-response trend is one of the more important factors when making assessments of causation in epidemiologic literature. These issues are addressed in greater detail in the accompanying comments by Michael Kelsh and Dominic Alexander.

Thus, based on an overall WOE analysis of the epidemiologic research, these data do not support the conclusion that there is "strong" or "convincing" evidence of a causal association between human exposure and cancer.

The EPA's 2005 guidelines also state that a chemical may be described as carcinogenic to humans with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence, all of which must be met. One of these lines of evidence is "extensive evidence of carcinogenicity in animals." Therefore, we now turn to an evaluation of the animal data.

In weighing the evidence in experimental animals and addressing the impact of the metabolites produced, EPA states that

> "A greater variability of response is expected than from exposure to a single agent making it particularly important to look at the TCE database in a holistic fashion rather than the results of a single study, especially for quantitative inferences." (EPA, page 4-233)

We agree with EPA that the database needs to be viewed holistically. EPA goes on to surmise that evidence for cancer is found in two species (rats and mice) and for more than one tumor endpoint (kidney, liver, lung and immune system). However, EPA's description of this evidence is unconvincing when starting from the neutral question of: "Does TCE cause cancer in experimental animals?" Of the 4 primary tissues that EPA evaluates for carcinogenicity, only one or perhaps two of them, liver and lung tumors in mice, rises to the level of biological significance. Discussion of the remaining tumor types appears to presuppose that TCE is carcinogenic. The resulting text appears then to overly discount negative data, of which there are many, and to highlight marginal findings. The text does not appear to be a dispassionate rendering of the available data.[1]

[1] For example, EPA (page 4-261) states "For rats, Maltoni et al. (1986) reported 4 liver angiosarcomas (1 in a control male rat, 1 both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and 1 in a

Specifically, EPA's conclusion that kidney cancer is evident in rats rests on one statistically significant finding in over 70 dose/tumor endpoint comparisons and references to exceedances of historical control values (NTP, 1990). Using a 0.05 p-value for statistical significance, a frequency of 1 or even several statistically or biologically significant events is expected in such a large number of dosed/tumor groups. This expectation is met, but not exceeded, as shown in Tables 1 and 2, which present the percent response for the various studies of kidney tumors, grouped by exposure level. EPA notes several other occurrences of kidney tumors, but the incidence was either not statistically significant or of borderline significance in comparison with concurrent controls. The presentation of data vs. the historical NTP controls is very useful. But historical control data needs to be presented in the context of both the study and year, since drift occurs in animal colonies (e.g., it is likely that the historical control data were different for the NCI 1976 study than for the NTP 1988-1990 studies). At least as importantly, historical control data is needed for each strain, particularly in light of the relatively high control response (7% in the inhalation study in Han:Wistar rats (Henschler et al., 1980). The statements about consistent increases of a rare tumor seem to assume that the background for all strains is the same as that reported by NTP for F344 rats. Moreover, each of the studies EPA cites has

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female rat exposed to 600-ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular "hepatomas" in treated and control rats were not given. Although Maltoni et al. (1986) concluded that the small number was not treatment related, ***the findings were brought forward*** [emphasis added] because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials." Perhaps we missed them in EPA's tome, but these data were not shown.

Another example of this tendency to discount negative findings is found on Page 4-263. "Although the mice in the two experiments [Maltoni et al., 1988, Table 4-55, page 4-258] in males were of the same strain, the background level of liver cancer was significantly different between mice from the different sources (1/90 versus 19/90), though the early mortality may have led to some censoring." Perhaps we missed EPA's point, but it appears that the Table 4-55 only presented one of the two control groups. Inclusion of the control group with the higher background level would suggest that there was no chemical-related increase.

problems. Although EPA generally does a good job of identifying these problems, its overall conclusion, based on these flawed studies cannot be that TCE is a known kidney tumorigen. The best that can be said is that the data are inconsistent.

EPA states that liver tumors are statistically significant in mice. This statement is confirmed by a biological judgment of all available data as shown in Tables 5 and 6.[2]

EPA finds three statistically significant occurrences of lung tumors in mice, 1 of them in a study with known epichlorohydrin contamination. Findings in other studies might be considered as biologically significant (see highlights in Tables 9 and 10 of these comments). The rest of the studies show no statistically significant increase, or show no lung tumors, or show a decrease in lung tumors as shown in Tables 7, 8, 9 and 10. Briefly, these data are either equivocal or marginally positive. EPA might consider revising its lung tumor table (Table 4-73) in order to make this information more readily transparent.

EPA states on page 4-397 that:

> "Cancers of the immune system that have been observed in animal studies and are associated with TCE exposure are summarized in Tables 4-68 and 4-69. The specific tumor types observed are malignant lymphomas, lymphosarcomas, and reticulum cell sarcomas in mice and leukemias in rats…

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[2] EPA (page 4-261) also states that "The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F1 mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related non-neoplastic liver lesions in males and a decrease in basophilic cytological change reported from TCE-exposure in female rats. **The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error [emphasis added].**

Note well, however, that NTP (1990) is the same study in which the ***sole*** statistically significant finding of kidney cancer in rats was made by EPA (page 4-179, Table 4-41). Thus, EPA appears to accept the findings of NTP (1990) when the result is positive (kidney), but not when the result is negative (liver).

EPA then continues on page 4-399 with:

> "In summary, overall there is limited available data in animals on the role of TCE in lymphomas and leukemias. There are few studies that analyze for lymphomas and/or leukemias. Lymphomas were described in four studies (NTP, 1990; NCI, 1976; Henschler et al., 1980, 1984) but study limitations (high background rate) in most studies make it difficult to determine if these are TCE-induced. Three studies found positive trends in leukemia in specific strains and/or gender (Maltoni et al., 1986, 1988; NTP, 1988). Due to study limitations, these trends cannot be determined to be TCE-induced."

In reading the text between these two apparently disparate quotes, the data for these cancers is overwhelmingly negative; some data might be statistically significant negative (Henchler et al., 1984). The use of EPA (2005) would suggest that these experimental animals findings are negative.

As currently written, the best argument that EPA can make with these experimental animal data is that the data provide **suggestive evidence of carcinogenicity**. A holistic viewpoint, one that EPA espouses, limits the interpretation and reliability of the animal data, and/or decreases the weight of evidence for carcinogenicity in rodents. Based on these considerations, the animal data for these four tumors do not meet the criterion of "extensive evidence of carcinogenicity in animals." Multiple marginal findings do not constitute "extensive evidence." We encourage EPA to either revise its text, with appropriate supporting data, to support a judgment of "likely to cause cancer in humans," or reconsider its conclusion based on these experimental animal data.

The epidemiologic literature on TCE can be characterized by many of the terms used to describe characteristics of the "suggestive" descriptor. These include the findings of a small increase in risk of tumors (kidney, NHL, liver) combined with the possibility that these cancers can be attributable to other known and unknown factors, and where there are studies that report positive responses, the limitations in study power, design, or conduct limit the ability to draw

"confident" conclusions. As shown in the data extracted from IRIS and presented in Table 11, the epidemiological data supporting a conclusion of "known" human carcinogen, or "A carcinogen" for other chemicals under the 1986 guidelines, is typically much stronger than the data for TCE.

The available experimental animal evidence can be interpreted in various ways depending on how EPA chooses to revise its text. As currently written, this evidence is primarily negative or conflicting for kidney and immune tumors, and positive for mouse liver tumors and lung tumors, and thus the overall weight of evidence considering both epidemiology and experimental animal evidence would be best seen as "suggestive." However, a more complete presentation and analysis of the animal data may push the overall classification into the "likely" category based on a "suggestive" characterization of the epidemiologic literature and consideration of the weight of evidence from the animal tumor data, particularly the data on liver tumors in mice.

However, in no circumstance is it scientifically reasonable to judge that TCE is "carcinogenic to humans" based on the available human and experimental animal data.

In summary, a review of the available epidemiologic evidence and related meta-analyses, and the experimental animal data as presented in the document indicate "**suggestive evidence of carcinogenic potential**" of TCE based on the EPA cancer guidelines. The overall database may indicate that TCE is at the low end of "likely human carcinogen," but the document as written does not currently make that case. Description of TCE as a known human carcinogen is precluded by:

- Methodological and analytical inconsistencies in the epidemiologic literature, such as weak summary associations, differences in results by sub-groups, lack of evidence of

dose-response relationships or insufficient data to fully evaluate exposure trends, and the potential influence of confounding by lifestyle or occupational factors.

Description of TCE as a likely carcinogen based on the draft EPA text is:

- Downweighted by the conflicting or negative experimental animal data for kidney and immune tumors, and weakly supported by the positive findings for mouse liver and lung tumors.
- EPA could improve its determination of kidney tumors findings by conducting a complete historical control analysis for each study that it deems scientifically credible, but it will need to re-evaluate NTP 1990 to determine whether this study meets these criteria. EPA should not discount the negative findings for NTP (1990) for rat liver tumors, but then accept the same study for findings of rat kidney tumors.[2]

**References**

Alexander DD, Kelsh MA, Mink PJ, Mandel JH, Basu R, Weingart W. A meta-analysis of occupational trichloroethylene exposure and liver cancer. Int Arch Occup Environ Health 2007; 81(2):127–143.

Alexander DD, Mink PJ, Mandel JH, Kelsh MA. A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukemia. Occup Med 2006; 56(7):485–93.

Charbotel B, Fevotte J, Hours M, martin J, Beregeret A. Case-Control Study on Renal Cell Cancer and Occupational Exposure to Trichloroethylene. Part II: Epidemiological Aspects. Ann.Occup.Hyg. 2006.

Kelsh MA, Alexander DD, Mink PJ, Mandel JH. Occupational trichloroethylene exposure and kidney cancer: a meta-analysis. Epidemiology. 2010 Jan;21(1):95-102.

Mandel JH, Kelsh MA, Mink PJ, Alexander D, Kalmes RM, Weingart M, Yost L Goodman M. Occupational trichloroethylene exposure and non-Hodgkins lymphoma: A meta-analysis and review. Occup Environ Med 2006; 63(9):597–607.

Raaschou-Nielsen O et al. Cancer risk among workers at Danish companies using trichloroethylene: a cohort study. Am.J.Epidemiol. 2003;158:1182-92.

U.S. Environmental Protection Agency. 2005. Guidelines for carcinogen risk assessment. Washington D.C. EPA/630/P-03/001B.

Table 11. Summary of the Number of Positive and Negative Studies for "Known" or "A" Human Carcinogens

| Chemical (Year of Assessment) | Epi Positive[1] | Epi Negative[2] | Animal Positive | Animal Negative | Rare[3] |
|---|---|---|---|---|---|
| Arsenic, inorganic (1994) | 14 | ND | 1 | 4 | N |
| Asbestos (1987) | 7(9) | 1 | 3 | 3 | Y (mesothelioma) |
| Benzene (1998) | 5(11)[4] | ND | -[4] | - | N |
| Benzene (2000 oral) | -[4] | - | 4(9) | ND | N |
| Benzene (1998 inhalation) | - | - | 3(5) | ND | N |
| Benzidine (1986) | 5 | ND | 1 | 4 | N |
| Bis (chloromethyl) ether (BCME) (1988) | 6 | ND | 1 | 4 | N |
| Chloromethyl methyl ether (CMME) (1987) | 9 | ND | 3 | 4 | N |
| Chromium (VI) (1998) | 25(30) | ND | 5 | 8 | Y |
| Coke oven emissions (1989) | 6(8) | 2 | 2 | 2 | Y |
| Nickel Refinery Dust (1987) | 6 | ND | 1 | 9 | N |
| Nickel subsulfide (1987) | 5 | 1 | 2 | 4 | Y |

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|---|---|---|---|---|---|
| Vinyl Chloride (2000) | 11(16) | 2 | 8(10) | 6(8) | Y (angio-sarcoma) |
| 1,3-Butadiene (2001) | 7(9) | ND | 1 | 1 | N |

[1] First number is the best estimate of number of unique cohorts, based on the IRIS summary. The number in parentheses is total number of citations of studies.

[2] ND = not determinable from writeup; no studies were mentioned, but it is not clear from the writeup whether negative studies exist, but were not included because a strength of evidence approach was in use at the time.

[3] Tumor associated with the chemical exposure has a very low background in humans, increasing the specificity of the association.

[4]There is one IRIS assessment for benzene, with portions from 1998 and 2000. The human data are presented in the initial 1998 assessment, while inhalation data for animals were presented in the 1998 document, and oral animal data presented in a 2000 document.

**Contaminated Water Supplies at Camp Lejeune,**
**Assessing Potential Health Effects**
**National Research Council of the National Academy of Sciences (2009)**

**BOX 1** Five Categories Used by IOM to Classify Associations

*Sufficient Evidence of a Causal Relationship*

Evidence from available studies is sufficient to conclude that a causal relationship exists between exposure to a specific agent and a specific health outcome in humans, and the evidence is supported by experimental data. The evidence fulfills the guidelines for sufficient evidence of an association (below) and satisfies several of the guidelines used to assess causality: strength of association, dose-response relationship, consistency of association, biologic plausibility, and a temporal relationship.

*Sufficient Evidence of an Association*

Evidence from available studies is sufficient to conclude that there is a positive association. A consistent positive association has been observed between exposure to a specific agent and a specific health outcome in human studies in which chance and bias, including confounding, could be ruled out with reasonable confidence. For example, several high-quality studies report consistent positive associations, and the studies are sufficiently free of bias, including adequate control for confounding.

*Limited/Suggestive Evidence of an Association*

Evidence from available studies suggests an association between exposure to a specific agent and a specific health outcome in human studies, but the body of evidence is limited. . . .

*Inadequate/Insufficient Evidence to Determine Whether an Association Exists*

Evidence from available studies is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

*Limited/Suggestive Evidence of No Association*

Evidence from well-conducted studies is consistent in not showing a positive association between exposure to a specific agent and a specific health outcome after exposure of any magnitude. . . .

Source: IOM (Institute of Medicine). 2003. Gulf War and Health, Vol. 2, Insecticides and Solvents. Washington, DC: National Academies Press.

## Contaminated Water Supplies at Camp Lejeune, Assessing Potential Health Effects National Research Council of the National Academy of Sciences (2009)

**BOX 2** Categorization of Health Outcomes[a] Reviewed in Relation to TCE, PCE, or Solvent Mixtures

*Sufficient Evidence of a Causal Relationship*

- No outcomes

*Sufficient Evidence of an Association*

- No outcomes

*Limited/Suggestive Evidence of an Association*

- Kidney cancer
- Adult leukemia (solvent mixtures)
- Multiple myeloma (solvent mixtures)
- Myleodysplasic syndromes (solvent mixtures)
- Scleroderma (solvent mixtures)
- Neurobehavioral effects (solvent mixtures)

*Inadequate/Insufficient Evidence to Determine Whether an Association Exists*

- Oral/pharyngeal cancer
- Nasal cancer
- Laryngeal cancer
- Esophageal cancer (TCE)
- Stomach cancer
- Colon cancer
- Rectal cancer
- Pancreatic cancer
- Hepatobiliary cancer
- Lung cancer (TCE)
- Bone cancer
- Soft tissue sarcoma
- Melanoma
- Non-melanoma skin cancer
- Breast cancer (TCE)
- Cervical cancer
- Ovarian/uterine cancer
- Prostate cancer
- Bladder cancer (TCE)
- Cancer of the brain or central nervous system
- Non-Hodgkin lymphoma
- Hodgkin disease
- Multiple myeloma
- Adult leukemia
- Myelodysplasic syndromes
- Childhood leukemia
- Childhood neuroblastoma
- Childhood brain cancer
- Aplastic anemia
- Congenital malformations
- Male infertility
- Female infertility (after exposure cessation)
- Miscarriage, preterm birth, or fetal growth restriction (from maternal preconception exposure or paternal exposure)
- Preterm birth or fetal growth restriction (from exposure during pregnancy)
- Cardiovascular effects
- Liver function or risk of cirrhosis
- Gastrointestinal effects
- Renal toxicity
- Amyotrophic lateral sclerosis
- Parkinson disease
- Multiple sclerosis
- Alzheimer disease
- Long-term reduction in color discrimination
- Long-term hearing loss
- Long-term reduction in olfactory function

*Limited/Suggestive Evidence of No Association*

- No outcomes

[a] *Outcomes for TCE and PCE unless otherwise specified**

* PCE-only outcomes omitted

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I have been asked to comment on the IRIS Document on trichloroethylene (TCE) by the Halogenated Solvents Industry Alliance. My laboratory has published extensively on the biotransformation of TCE and was among the first to report formation of glutathione-*S*-conjugates from TCE. My area of expertise is biotransformation of xenobiotics, mechanisms of toxicity, and genotoxicity testing and I have published more then 180 manuscripts in these areas. Moreover, I am/was member of several advisory panels charged with health risk assessment of chemicals including the European Union Scientific advisory committee on Health and Environment (SCHER). As a member of this committee, I was the lead author of the review of the European Chemicals Bureau risks assessment report on TCE. I also have followed the many controversies in the risk assessment of TCE over the last 30 years.

## General comments

The toxicity database on TCE is very large, with a number of controversial areas relevant to health risk assessment. EPA has generated a large document and attempted to comprehensively cover the available toxicology information on TCE and its metabolites. Most of the available studies are covered by the assessment. However, the document would have benefited from a detailed evaluation of the strengths and weaknesses of the individual studies and a selection of key studies based on a weight of evidence approach. In several places in the document, study results are just reiterated and some of the conclusions relevant for deriving RfDs and RfCs have apparently been taken from reviews. A detailed justification based on evaluation of the individual studies and a consideration of controversial data not supporting conclusions by EPA is often insufficiently developed. Identical criteria should be applied to the level of evidence required to support or discount a mode of action (MoA).

## Specific comments:

### 1. Extent of glutathione *S*-conjugate formation from TCE

The document concludes that the extent of formation of *S*-(1,2-dichlorovinyl)glutathione (DCVG) from TCE in humans is much higher as compared to rodents. Since this conclusion has a major impact on the derivation of RfCs and RfDs for TCE, it should be well justified and based on consideration of all available data. Apparently, EPA supports

this conclusion with high blood concentrations of DCVG reported in humans after inhalation of TCE (Lash *et al.*, 1999b). This observation is in contrast to the very low concentrations of the isomers of N-acetyl-S-(1,2-dichlorovinly)-L-cysteine (N-acetyl-DCVC) in urine. The consideration of this dataset without the wealth of other information therefore suggests that which therefore can not be a quantitative biomarker of metabolic flux through the glutathione conjugation pathway (Lash *et al.*, 2000) and that most of the DCVG may undergo bioactivation by ß-lyase. However, a number of observations do not support this conclusion:

- In the human study with TCE inhalation, high concentrations of DCVG were indicated using a complex analytical procedure, often called the "Reed-Method" (Reed *et al.*, 1980). This method was developed to determine low concentrations of glutathione and glutathione disulfide and may be used to quantify DCVG formation in biological samples. The method involves reaction of the thiol with iodoacetamide and the amino group with chlorodinitrobenzene, followed by ion exchange chromatography and UV-detection of the dinitrophenyl chromophore. Due to the ion-exchange chromatography with a high salt concentration in the eluate, retention times shifts are common due to column deterioration (Lash *et al.*, 1999b). Since the method is not selective for DCVG and analysis of biological samples produces many peaks, retention time shifts may create problems to locate the DCVG peak.

A number of inconsistent datasets questions the reliability of the "Reed-method" to determine DCVG and DCVC:

- In a study assessing DCVG and DCVC formation in rodents after high oral doses of TCE, DCVG-concentrations reported in blood were high, but did not show dose or time-dependence (Lash *et al.*, 2006). In addition, the study reports high concentrations of DCVC excreted in urine. EPA calls the results of this study "aberrant", but apparently did not further assess reliability. Others have reported a very low rate of DCVC-formation in vivo (Dekant *et al.*, 1990; Kim *et al.*, 2009) and DCVC has not been reported as urinary metabolite of TCE using either mass spectrometry or HPLC which radiochemical detection after administration of $^{14}$C-TCE (Dekant *et al.*, 1986a).
- The "Reed-method" has also been used to determine DCVG-formation from TCE in subcellular fractions from liver and kidney of rats, mice, and humans. Again, high rates of formation of DCVG were reported (table 1). In contrast, using $^{14}$C-TCE and radioactivity detection, much lower reaction rates were observed in other studies (table 1). In addition, isolated glutathione *S*-transferases also have a very low capacity to metabolize TCE to DCVG (Hissink *et al.*, 2002) and the application of the "Reed-method" to study formation of *S*-(1,2,2-trichlorovinyl)glutathione (TCVG) from perchloroethylene in subcellular fractions also gave much higher rates of formation (Lash *et al.*, 1998) as compared to methods using $^{14}$C-perchloroethylene and HPLC with radioactivity detection (Dekant *et al.*, 1987; Green *et al.*, 1990; Dekant *et al.*, 1998).

Therefore, DCVG concentrations determined by the "Reed-method" may be widely overestimated. The more reliable and consistent data support a very low extent of DCVG formation in rodents:

- Very low rates of formation of DCVG in rodents liver subcellular fractions are consistent with very low blood levels of DCVG in mice (Kim *et al.*, 2009) and a very low biliary elimination of DCVG in rats after oral administration of doses > 2 000 mg TCE/kg bw (Dekant *et al.*, 1990). In mice, DCVG concentrations were several 1,000-fold lower than those of the oxidative metabolite trichloroacetic acid (TCA) (Kim *et al.*, 2009). In rats, biliary elimination of DCVG within seven hours after oral administration was 2 microg and accounted for << 0.01 % of administered dose (Dekant *et al.*, 1990). Due to its

molecular weight (> 350 D) and the presence of effective transport systems for glutathione *S*-conjugates in the canalicular membrane, most of the DCVG formed in rat liver is expected to be excreted with bile. Therefore, the low concentrations of DCVG in blood of mice and the low recovery of DCVG in bile of rats after TCE-administration well support very low rates of DCVG formation.

- Even when considering the high rates of DCVG formation reported in subcellular fractions and the only 3-fold difference in reaction rates between mouse, rat and humans (table 1), it is difficult to explain why DCVG-blood levels in mice after a very high oral dose are orders of magnitude lower than those reported in humans after inhalation exposures giving a much lower internal TCE-dose.
- High blood concentrations of DCVG and a high flux through ß-lyase bioactivation are not consistent with the human toxicity data on TCE. Despite high occupational exposures to TCE between the 1950s and 1970s (occupational exposure limits for TCE were 200 ppm in Germany and were often exceeded for prolonged times), overt nephrotoxicity was rarely observed even after many years of exposures (MAK, 1996). Using the blood concentrations reported and extrapolating to a daily exposure to 200 ppm TCE for 8 h, daily doses of DCVC of app. 5-7 mg/kg bw should have been received by workers. A significant flux through ß-lyase bioactivation should have resulted in renal effects considering the alleged potency of DCVG.
- Kinetic studies on acetylation, and ß-lyase-mediated metabolism of DCVC support a low flux through ß-lyase activation since the relative flux through the *N*-acetylation pathway (detoxication) is one to two orders of magnitude higher then through ß-lyase activation (Green *et al.*, 1997a). In addition, a low flux through ß-lyase is indicated by the recovery of most of a low intravenous dose of DCVC isomers in urine as mercapturic acids in rats (Birner *et al.*, 1997), the weak nephrotoxicity of DCVC (Green *et al.*, 1997a) and observations with perchloroethene, which is also metabolized by glutathione *S*-conjugate formation and ß-lyase. The perchloroethylene (PERC) metabolite *S*-(1,2,2-trichlorovinyl)-L-cysteine is cleaved by ß-lyase to dichloroacetic acid (DCA) which, when formed in the kidney, is excreted with urine. While DCA is a metabolite of PERC in rats, this compound is not excreted as PERC metabolite in humans (Völkel *et al.*, 1998). In addition, dichloroacetylated proteins were detected both in rat kidney proteins and rat blood proteins after PERC inhalation. Such protein modifications were not detected in blood proteins from humans after identical exposures (Pähler *et al.*, 1999). These observations indicate that flux through ß-lyase in humans is even lower as compared to rodents.
- Chloracetic acid is formed by ß-lyase from DCVC (Dekant *et al.*, 1988). In rodents, chloroacetic acid and its metabolites (Green and Hathway, 1975; Green and Hathway, 1977) are not significant metabolites of TCE (> 0.1 % of radioactivity in urine) (Dekant *et al.*, 1984; Dekant *et al.*, 1986a). If the ß-lyase pathway is more relevant, such metabolites should be present in urine in higher concentrations. Other metabolites indicative of alternative processing of DCVC have also not been detected in humans (Bloemen *et al.*, 2001).

In summary, the assumption of a major flux through glutathione S-conjugate formation in TCE metabolism both in humans and in rodents is not well supported.

Table 1: Reported rates of formation of DCVC from Trichloroethene (TCE) in rat, mouse and human subcellular fractions. The concentration of TCE in the incubation is based on the amount added.

| Tissue | Species | TCE Conc (mM) | Rate of DCVC formation (pmol/minxmg) | Analytical method to determine DCVG | Reference |
|---|---|---|---|---|---|
| Liver cytosol | Rat | 1.4 (14C) | 0.54 (non-enzymatic reaction rates substracted) | HPLC with radiochemical detection, peak identity confirmed by LC/MS | (Green *et al.*, 1997b) |
| | Mouse | 1.9 (14C) | 0.35 | | |
| | Human | 1.9 – 2.5 (14C) | 0.012 – 0.055 | | |
| Liver microsomes | Rat | 1.4 (14C) | Not different from non-enzymatic reaction | | |
| | Mouse | 1.9 (14C) | n.d. | | |
| | Human | 1.9 – 2.5 (14C) | n.d. | | |
| Kidney cytosol | Rat | 1.4 (14C) | Not different from non-enzymatic reaction | | |
| | Mouse | n.d. | | | |
| | Human | n.d. | | | |
| Kidney microsomes | Rat | 1.4 (14C) | Not different from non-enzymatic reaction | | |
| | Mouse | n.d. | | | |
| | Human | n.d. | | | |
| Liver cytosol | Rat | 4 (14 C) | < 2 | HPLC with radioactivity detection, peak identity confirmed by GC/MS after hydrolysis | (Dekant *et al.*, 1990) |
| Liver microsomes | Rat | 4 (14C) | 2 | | |
| Liver cytosol | Rat | 2 | 121 (males)<br>81 (females) | Derivatisation with DNCB and ion exchange HPLC | (Lash *et al.*, 1999a) |
| | Mouse | 2 | 408 (males)<br>361 (females) | | |
| | Human | 1 | 1 700 – 4 180 | | |
| Liver microsomes | Rat | 2 | 171 (males)<br>120 (females) | | |
| | Mouse | 2 | 666 (males)<br>426 (females) | | |
| | Human | 1 | 495 – 3 245 | | |
| Kidney cytosol | Rat | 2 | 7.5 (males)<br>5.3 (females) | | |
| | Mouse | 2 | 93 (males)<br>61 (females) | | |
| | Human | na | 810 (vmax) | | |
| Kidney microsomes | Rat | 2 | Nd (males)<br>1.0 (females) | | |
| | Mouse | 2 | 91 (males)<br>278 (females) | | |
| | Human | na | 6 290 (vmax) | | |

## 2. The role of glutathione *S*-conjugates in nephrotoxicity and renal tumor formation by TCE

Since *S*-conjugates of TCE are nephrotoxic in rodents and genotoxic in vitro, it is appealing to conclude that *S*-conjugate formation is involved in nephrotoxicity of TCE and that the MoA for kidney tumor formation is genotoxicity. However, a number of contradictory findings are not adequately considered in the IRIS-document.

- Formation rates for DCVC in subcellular fractions from mice and rats are similar (or even higher in mice) suggesting similar doses of DCVC to the kidney in both species (Green *et al.*, 1997a; Kim *et al.*, 2009). Moreover, activation of TCE by the ß-lyase pathway is higher in mice (Eyre *et al.*, 1995), DCVC is more nephrotoxic in mice, and causes higher rates of cell replication and covalent binding in mice as compared to rats (Eyre *et al.*, 1995; Green *et al.*, 1997a). Yet, mice are not sensitive to TCE induced renal tumor formation.
- Based on the nephrotoxicity of DCVC and the low rates of formation of DCVC both in rats and mice in vivo, it is questionable if the very low concentrations of DCVG formed in rodents can explain nephrotoxicity and tumor formation. Extrapolating the DCVG blood concentrations observed after single doses to the doses applied in the carcinogenicity studies, daily DCVC-doses in the two year studies were less than 0.03 mg/kg bw. This is orders of magnitude below the doses of DCVC required to induce nephrotoxicity (Terracini and Parker, 1965) and questions an involvement of this pathway in nephrotoxicity.
- EPA concludes that trichloroethanol and formic acid formation may not be involved in the toxicity of TCE to the kidney due to differences in pathology observed between TCE and trichloroethanol treated rats. In my opinion, such comparisons are difficult since differences in the kinetic profiles of a compound formed as a metabolite or administered per se are likely major confounders.
- EPA states that data on VHL gene mutations support a mutagenic MoA in TCE-induced kidney tumors. This is based on studies (Bruning *et al.*, 1997; Brauch *et al.*, 2004) reporting VHL mutations in renal tumors of TCE-exposed individuals. It is concluded that comparison of TCE-exposed and non-exposed patients (Brauch *et al.*, 2004) revealed clear differences with respect to (1) frequency of somatic VHL mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. As discussed in Brauch et al. (2004), the mutation frequency in the non-exposed patients (10%) was considerably lower than that commonly observed in sporadic renal tumors, e.g. 82.4% (Nickerson *et al.*, 2008) or 71% in (Banks *et al.*, 2006), and technical problems using archived tissue samples may be the cause. Given that exon 3, which harbors the multiple mutations seen in TCE exposed patients, did not amplify in most of the controls, there is limited evidence for a difference in the incidence of multiple mutations and frequency of somatic VHL mutations, although the C454T transition appears to be characteristic of tumors in TCE exposed patients. However, the presence of mutations in human tumors does not lead to the conclusion that VHL mutations occur early during carcinogenesis and hence are no evidence for direct genotoxicity of TCE. In contrast, experimental data in rats show that neither TCE nor its active metabolite DCVC induce VHL mutations (Mally *et al.*, 2006), suggesting that VHL mutations in humans may be acquired at later stages of tumor development. While the document argues that the VHL gene may not be a target gene in rodent models of renal carcinogenesis, only few studies have looked at VHL in rats and there is no support for the hypothesis that the role of VHL is different in rats and humans.

- The Eker rat may be an useful rodent model for renal cell carcinoma (RCC), but the molecular basis for chemically induced tumor formation in rats and RCC in humans may be widely different from spontaneous tumor formation in this rat strain, as high-grade RCCs can develop in the absence of mutations in the Tsc2 gene in rats (Toyokuni *et al.*, 1998). Development of high-grade renal cell carcinomas in rats independently of somatic mutations in the Tsc2 and VHL tumor suppressor genes (Toyokuni *et al.*, 1998) demonstrates that mutational inactivation of TSC2 or VHL is not a prerequisite for renal carcinogenesis. The similar pathway activation in Eker rat RCC as that seen in humans with VHL mutations reported (Liu *et al.*, 2003) involves deregulation of HIFalpha and VEGF expression which frequently occur in various cancers and provide little evidence to suggest that Tsc-2 inactivation in rats is "analogous" to inactivation of VHL in human RCC.
- Epidemiological data may support an association between specific VHL mutations and TCE exposure, this does not indicate an early event in RCC and – in the absence of experimental support - should not be taken as support for a mutational MoA.
- EPA uses a micronucleus/comet assays data in rat kidney after TCE-administration as support for a genotoxic MoA. However, the positive micronucleus (Robbiano *et al.*, 2004) assay applied a very high dose and used an inappropriate route of administration (ip injection of ½ of the $LD_{50}$). Due to the high dose applied and the route of administration, the results may be confounded by inflammatory responses and should not be used for conclusions. A comet assay in the kidney using repeated inhalation exposures to TCE was negative (Clay, 2008). The decision to not use this study in the assessment is insufficiently justified. The inhalation study used a higher number of animals (5/group) as compared to the ip study, which states $n > 3$ with an apparent maximum of 5. The comet assay also shows that administered DCVC is only weakly active in the kidney.
- EPA argues that there is no link between nephrotoxicity and renal tumor formation. However, there are a number of compounds causing renal tumors in rats without being genotoxic. For example, cytotoxicity and regenerative cell proliferation (Swenberg and Lehman-McKeeman, 1999) is accepted as MoA for $\alpha_{2u}$-globulin binding agents (TCE does not bind to $\alpha_{2u}$-globulin, but may also causes tumors through nephroxicity).

**3. Mode of action for liver carcinogenesis**

- EPA spends considerable effort to correlate liver tumor induction by TCE in mice with liver tumor induction observed after administration of the TCE metabolites TCA and DCA. Again, such comparisons are inherently complex. Both DCA and TCA were administered with drinking water and TCE studies applied gavage in oil. The different administration regimens will result in different time courses of the administered compounds or metabolites in blood and dose-dependent bioavailability may further complicate the interpretation.
- It is highly questionable that DCA is involved in liver tumor induction by TCE since it is only formed in very low concentrations from TCE in rodents (Dekant *et al.*, 1986a; Kim *et al.*, 2009). In mice, DCA is formed in concentrations several orders of magnitude below those of TCA. Thus, DCA would be required to be a highly potent liver carcinogen, which it is not. Therefore, the potency data on DCA do not suggest that the high liver tumor incidence induced by TCE in mice is related to DCA formation. In

addition, DCA is not a human urinary metabolite of TCE (Bernauer *et al.*, 1996; Bloemen *et al.*, 2001).

- With TCA, EPA derives a dose-dependence from tumor incidence data in drinking water studies. Apparently, EPA assumes a dose-independent high bioavailability of TCA. However, the oral bioavailability of TCA from drinking water is limited, concentration-dependent and significantly reduced at higher concentrations of TCA (Larson and Bull, 1992; Templin *et al.*, 1993; Sweeney *et al.*, 2009). The incidence data therefore need to be corrected to account for the limited bioavailability of TCA at higher concentrations in drinking water.
- The mostly negative data in mutagenicity testing with TCE using liver specific activation and negative in vivo gentoxicity data including a very low DNA-binding in liver of mice (Bergman, 1983; Kautiainen *et al.*, 1997) also do not support a mutagenic MoA for liver tumors. Due to intensive metabolism by oxidation and reduction, chloral hydrate concentrations in the liver are low, chloral hydrate is a very weak mutagen. Therefore, chloral hydrate mutagenicity cannot adequately explain the formation of liver tumors by TCE in mice.
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## 4. Mode of action for lung tumorigenesis.

EPA considers the lung tumors induced by TCE in specific strains of mice as relevant to humans and implies a genotoxic mode-of action. EPA tries to devaluate the hypothesis that chloral may reach high concentrations in mouse lung cells. However, the arguments by EPA are not convincing.

Rat and guinea pig data should not be used to conclude on biotransformation in mouse lung.

- A delivery of TCE from the systemic circulation in mice also causes lung toxicity due to the high metabolic capacity in the target cell. If TCE-metabolites formed in the liver are transported to the lung to cause toxicity there, the species-specificity is difficult to explain since the same metabolites are also present in rats, which do not show lung toxicity.
- A high rate of chloral formation from TCE and limited capacity for further metabolism of chloral (low capacity for reduction of chloral hydrate to trichloroethanol, low capacity for conjugation of trichloroethanol) will result in much higher steady state levels of chloral hydrate in mouse lung Clara cells as compared to rat or human lung (Odum *et al.*, 1992; Green *et al.*, 1997b). The high steady state levels may result in cytotoxicity.
- Cells damaged by the high chloral concentrations formed by TCE-metabolism initiate regeneration and replication to repair and replace the damaged Clara cells (Villaschi *et al.*, 1991) and repeated cycles of damage and regeneration may finally result in lung tumor formation.

Support for a cytotoxic MoA regarding the mouse lung tumors induced by TCE can also be derived from observations with other chemicals. The consequences of Clara cell specific cytotoxicity for tumor induction has been assessed with a number of other chemicals and the very high capacity of the mouse lung Clara cell for biotransformation is also the basis for the mouse-specific lung toxicity. The assessment therefore should integrate this information.

- Styrene, naphthalene, and coumarin induce lung tumors in mice and chronic damage of Clara cells including hyperplasia, often with a time- and dose-related increase in

bronchiolar hyperplasia in terminal bronchioles. As with TCE, lung lesions are induced by short term administration, recess after repeated exposures and reappear after continuing exposures. None of these chemical induced lung tumors or histopathologic changes in rat lung (Cruzan *et al.*, 1998; Cruzan *et al.*, 2001).

- Major species differences in lung tumor induction and lung anatomy are one likely basis for the selective tumorigenicity of these chemicals in mice. Lung tumors occur spontaneously in several mouse strains and the incidences of benign lung tumors in control mice are often very high. In general, murine lung tumors are mostly adenomas originating from bronchiolar Clara cells. The adenomas may progress to adenocarcinomas. (Witschi, 1991).
- Clara cells are the major site of xenobiotic metabolism in the mouse lung (Chichester *et al.*, 1991; Buckpitt *et al.*, 1995). In addition to marked species differences in metabolic capacity of Clara cells in different species, species differences in Clara cell abundance and function may contribute to selective pulmonary toxicity in mice. Clara cell number is significantly higher within the terminal bronchioles of mice relative to rats and humans (Plopper *et al.*, 1980; Lumsden *et al.*, 1984). Clara cells represent approximately 5 % of all cell types and are distributed throughout the airways in mice. In humans, only very few Clara cells are present and are localized in specific regions. Moreover, Clara cells differ morphologically among species, with human cells containing little smooth endoplasmic reticulum.
- TCE and the other chemicals inducing selective lung damage and lung tumors in mice require biotransformation by pulmonary CYP2F and CYP2E1 (Green *et al.*, 1997b; Shultz *et al.*, 1999; Shultz *et al.*, 2001; Born *et al.*, 2002; West *et al.*, 2002; Forkert *et al.*, 2005).
- In mice, both CYP2E1 and CYP2F1 are preferentially localized in Clara cells (Forkert *et al.*, 1989; Buckpitt *et al.*, 1995; Forkert, 1995; Shultz *et al.*, 2001). In rat lung, the expression of CYP2F4, an orthologe of mouse CYP2F2 (Baldwin *et al.*, 2004) is app. 30-fold lower consistent with a much lower turnover of CYP2F substrates in rat. Evidence for the presence of the the human orthologe CYP2F1 in human lung is lacking. In rhesus monkeys, CYP2F1 was not detected in the respiratory tract except in the nasal epithelium (Ding and Kaminsky, 2003; Baldwin *et al.*, 2004). CYP2E1 catalytic activity is present in human lung with an activity app. 100fold lower then in human liver (Bernauer *et al.*, 2006). In summary, the available information on the presence and catalytic activities of CYP2E1 and CYP2F enzymes in the lung of different species suggest a much higher activity of these enzymes in the mouse, the species susceptible to the pneumotoxicity.
- Studies directly quantifying relevant metabolite formation from the different pneumotoxic compounds and mice consistently have a much higher capacity for oxidation as compared to rats and humans. The available data on the mode-of-action for induction of lung tumors share many common features with regard to the induction of Clara cell lesions in the mouse and a number of observations support a non-genotoxic mode-of-action: Glutathione depletion is a major determinant of the toxic responses in the mouse Clara toxicity (West *et al.*, 2000a; West *et al.*, 2000b; Plopper *et al.*, 2001; Phimister *et al.*, 2004; Turner *et al.*, 2005). Glutathione-depletion induced cell death induced by mouse specific Clara cell toxicants initiates extensive cell replication and subsequent hyperplasia which are considered important steps in the multi-step progression to tumor development (Gadberry *et al.*, 1996; Green *et al.*, 1997b; Green *et al.*, 2001).
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## Additional comments

Page 2-22: Line 36, the exposures in the cardboard workers in Germany likely were much higher, with peaks well above 1,000 ppm and prolonged exposures above the former occupational standard (> 200 ppm TWA).

Page 3-6: The major toxicity of TCE after acute high dose exposure is narcosis. Both kidney and liver damage are not often observed (MAK, 1996).

Page 3-13: Table 3-6, if the data in the table are not considered reliable why are they presented?

Page 3-15: Line 27, TCA reversibly binds to proteins and the reversible protein binding is much more relevant for toxicokinetics of TCE as compared to covalent binding. It should also be noted that the $^{14}$C-TCE used in many of the early studies contained a number of reactive impurities.

Page 3-23: Regarding saturation of TCE metabolism in humans, none of the human studies used dose-ranges where saturation of metabolism was seen in rats. Therefore, this conclusion should be removed.

Page 3-24: Lines 9 to 14, the text is not logical. TCE oxide may rearrange to dichloroacetyl chloride and the TCE P450 intermediate may rearrange to give chloral (Miller and Guengerich, 1982; Liebler and Guengerich, 1983; Cai and Guengerich, 2001).

Page 3-25: Lines 20 to 23, TCE oxide does not rearrange to chloral. Therefore, the text is confusing.

Page 3-27, Lines 19 to 25, chloral hydrate as been identified as a circulating TCE metabolite and is also formed as the major product in the microsomal oxidation of TCE (Byington and Leibman, 1965; Cole *et al.*, 1975).

Page 3-35: Metabolite recovery data in male and female human beings are available. In addition, metabolite excretion in humans and rats exposed under identical conditions are available (Bernauer *et al.*, 1996).

Page 3-44: Table 3-23 should include additional data on GSH-conjugation of TCE (Dekant *et al.*, 1990; Green *et al.*, 1997a).

Page 3-46: Information on ß-lyase catalyzed metabolism of DCVC is available (Green *et al.*, 1997a).

Page 3-47: DCVC-sulfoxide, it should be mentioned that sulfoxides and down-stream metabolites have never been directly identified in rodents.

Page 4-34: Line 1, conclusion on bacterial mutagenicity. A more detailed weight-of-evidence evaluation of the contradictory database is needed here.

Table 4-18: Robbiano study, the study did not apply DCVG or DCVC and thus should not be included in the table.

Page 4-83: Line 28, DCVC is a "direct-acting" mutagen since bacteria express ß-lyase (Dekant *et al.*, 1986b). Thus, this is a difference when compared to *S*-(2-chlorethyl)-L-cysteine, which does not require enzymatic transformation.

Page4-443: Lines 6 -7, the reactivity of chloral hydrate and chloroacetaldehyde are highly different and should not be compared. Chloroacetaldehyde is highly reactive with DNA-constituents (Green and Hathway, 1978), whereas chloral hydrate has not.

## References


Baldwin, R. M., Jewell, W. T., Fanucchi, M. V., Plopper, C. G., and Buckpitt, A. R. (2004). Comparison of pulmonary/nasal CYP2F expression levels in rodents and rhesus macaque. *J Pharmacol Exp Ther* **309**, 127-136.

Banks, R. E., Tirukonda, P., Taylor, C., Hornigold, N., Astuti, D., Cohen, D., Maher, E. R., Stanley, A. J., Harnden, P., Joyce, A., Knowles, M., and Selby, P. J. (2006). Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer. *Cancer Res* **66**, 2000-2011.

Bergman, K. (1983). Interactions of trichloroethylene with DNA in vitro and with RNA and DNA of various mouse tissues in vivo. *Arch Toxicol* **54**, 181-193.

Bernauer, U., Birner, G., Dekant, W., and Henschler, D. (1996). Biotransformation of trichloroethene: dose-dependent excretion of 2,2,2-trichloro-metabolites and mercapturic acids in rats and humans after inhalation. *Arch Toxicol* **70**, 338-346.

Bernauer, U., Heinrich-Hirsch, B., Tonnies, M., Peter-Matthias, W., and Gundert-Remy, U. (2006). Characterisation of the xenobiotic-metabolizing Cytochrome P450 expression pattern in human lung tissue by immunochemical and activity determination. *Toxicol Lett* **164**, 278-288.

Birner, G., Bernauer, U., Werner, M., and Dekant, W. (1997). Biotransformation, excretion and nephrotoxicity of haloalkene-derived cysteine S-conjugates. *Arch Toxicol* **72**, 1-8.

Bloemen, L. J., Monster, A. C., Kezic, S., Commandeur, J. N., Veulemans, H., Vermeulen, N. P., and Wilmer, J. W. (2001). Study on the cytochrome P-450- and glutathione-dependent biotransformation of trichloroethylene in humans. *Int Arch Occup Environ Health* **74**, 102-108.

Born, S. L., Caudill, D., Fliter, K. L., and Purdon, M. P. (2002). Identification of the cytochromes P450 that catalyze coumarin 3,4-epoxidation and 3-hydroxylation. *Drug Metab Dispos* **30**, 483-487.

Brauch, H., Weirich, G., Klein, B., Rabstein, S., Bolt, H. M., and Bruning, T. (2004). VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference? *Toxicol Lett* **151**, 301-310.

Bruning, T., Weirich, G., Hornauer, M. A., Hofler, H., and Brauch, H. (1997). Renal cell carcinomas in trichloroethene (TRI) exposed persons are associated with somatic mutations in the von Hippel-Lindau (VHL) tumour suppressor gene. *Arch Toxicol* **71**, 332-335.

Buckpitt, A., Chang, A. M., Weir, A., Van Winkle, L., Duan, X., Philpot, R., and Plopper, C. (1995). Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. *Mol Pharmacol* **47**, 74-81.

Byington, K. H., and Leibman, K. C. (1965). Metabolism of trichloroethylene in liver microsomes. II. Identification of the reaction product as chloral hydrate. *Mol Pharmacol* **1**, 247-254.

Cai, H., and Guengerich, F. P. (2001). Reaction of trichloroethylene and trichloroethylene oxide with cytochrome P450 enzymes: inactivation and sites of modification. *Chem Res Toxicol* **14**, 451-458.

Chichester, C. H., Philpot, R. M., Weir, A. J., Buckpitt, A. R., and Plopper, C. G. (1991). Characterization of the cytochrome P-450 monooxygenase system in nonciliated

bronchiolar epithelial (Clara) cells isolated from mouse lung. *Am J Respir Cell Mol Biol* **4**, 179-186.

Clay, P. (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney. *Mutagenesis* **23**, 27-33.

Cole, W. J., Mitchell, R. G., and Salamonsen, R. F. (1975). Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. *J Pharm Pharmacol* **27**, 167-171.

Cruzan, G., Cushman, J. R., Andrews, L. S., Granville, G. C., Johnson, K. A., Bevan, C., Hardy, C. J., Coombs, D. W., Mullins, P. A., and Brown, W. R. (2001). Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. *J Appl Toxicol* **21**, 185-198.

Cruzan, G., Cushman, J. R., Andrews, L. S., Granville, G. C., Johnson, K. A., Hardy, C. J., Coombs, D. W., Mullins, P. A., and Brown, W. R. (1998). Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. *Toxicol Sci* **46**, 266-281.

Dekant, W., Berthold, K., Vamvakas, S., Henschler, D., and Anders, M. W. (1988). Thioacylating intermediates as metabolites of *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2,2-trichlorovinyl)-L-cysteine formed by cysteine conjugate ß-lyase. *Chemical Research in Toxicology* **1**, 175-178.

Dekant, W., Birner, G., Werner, M., and Parker, J. (1998). Glutathione conjugation of perchloroethene in subcellular fractions from rodent and human liver and kidney. *Chem Biol Interact* **116**, 31-43.

Dekant, W., Koob, M., and Henschler, D. (1990). Metabolism of trichloroethene - *in vivo* and *in vitro* evidence for activation by glutathione conjugation. *Chemico-Biological Interactions* **73**, 89-101.

Dekant, W., Martens, G., Vamvakas, S., Metzler, M., and Henschler, D. (1987). Bioactivation of tetrachloroethylene. Role of glutathione S-transferase-catalyzed conjugation versus cytochrome P-450-dependent phospholipid alkylation. *Drug Metab Dispos* **15**, 702-709.

Dekant, W., Metzler, M., and Henschler, D. (1984). Novel metabolites of trichloroethylene through dechlorination reactions in rats, mice and humans. *Biochem. Pharmacol.* **33**, 2021-2027.

Dekant, W., Schulz, A., Metzler, M., and Henschler, D. (1986a). Absorption, elimination and metabolism of trichloroethylene: a quantitative comparison between rats and mice. *Xenobiotica* **16**, 143-152.

Dekant, W., Vamvakas, S., Berthold, K., Schmidt, S., Wild, D., and Henschler, D. (1986b). Bacterial ß-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene. *Chemico-Biological Interactions* **60**, 31-45.

Ding, X., and Kaminsky, L. S. (2003). Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* **43**, 149-173.

Eyre, R. J., Stevens, D. K., Parker, J. C., and Bull, R. J. (1995). Acid-labile adducts to protein can be used as indicators of the cysteine S-conjugate pathway of trichloroethene metabolism. *J Toxicol Environ Health* **46**, 443-464.

Forkert, P. G. (1995). CYP2E1 is preferentially expressed in Clara cells of murine lung: localization by in situ hybridization and immunohistochemical methods. *Am J Respir Cell Mol Biol* **12**, 589-596.

Forkert, P. G., Baldwin, R. M., Millen, B., Lash, L. H., Putt, D. A., Shultz, M. A., and Collins, K. S. (2005). Pulmonary bioactivation of trichloroethylene to chloral hydrate: relative contributions of CYP2E1, CYP2F, and CYP2B1. *Drug Metab Dispos* **33**, 1429-1437.

Forkert, P. G., Vessey, M. L., Park, S. S., Gelboin, H. V., and Cole, S. P. (1989). Cytochromes P-450 in murine lung. An immunohistochemical study with monoclonal antibodies. *Drug Metab Dispos* **17**, 551-555.

Gadberry, M. G., DeNicola, D. B., and Carlson, G. P. (1996). Pneumotoxicity and hepatotoxicity of styrene and styrene oxide. *J Toxicol Environ Health* **48**, 273-294.

Green, T., Dow, J., Ellis, M. K., Foster, J. R., and Odum, J. (1997a). The role of glutathione conjugation in the development of kidney tumours in rats exposed to trichloroethylene. *Chemico-Biological Interactions* **105**, 99-117.

Green, T., and Hathway, D. E. (1975). The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem Biol Interact* **11**, 545-562.

Green, T., and Hathway, D. E. (1977). The chemistry and biogenesis of the S-containing metabolites of vinyl chloride in rats. *Chem Biol Interact* **17**, 137-150.

Green, T., and Hathway, D. E. (1978). Interactions of vinyl chloride with rat-liver DNA in vivo. *Chem Biol Interact* **22**, 211-224.

Green, T., Mainwaring, G. W., and Foster, J. R. (1997b). Trichloroethylene induced mouse lung tumours: studies of the mode of action and comparisons between species. *Fundamental and Applied Toxicology* **37**, 125-130.

Green, T., Odum, J., Nash, J. A., and Foster, J. R. (1990). Perchloroethylene-induced rat kidney tumors: an investigation of the mechanisms involved and their relevance to humans. *Toxicol. Appl. Pharmacol.* **103**, 77-89.

Green, T., Toghill, A., and Foster, J. R. (2001). The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity. *Toxicology* **169**, 107-117.

Hissink, E. M., Bogaards, J. J. P., Freidig, A. P., Commandeur, J. N. M., Vermeulen, N. P. E., and van Bladeren, P. J. (2002). The use of in vitro metabolic parameters and physiologically based pharmacokinetic (PBPK) modeling to explore the risk assessment of trichloroethylene. *Environmental Toxicology and Pharmacology* **11**, 259-271.

Kautiainen, A., Vogel, J. S., and Turteltaub, K. W. (1997). Dose-dependent binding of trichloroethylene to hepatic DNA and protein at low doses in mice. *Chem Biol Interact* **106**, 109-121.

Kim, S., Kim, D., Pollack, G. M., Collins, L. B., and Rusyn, I. (2009). Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. *Toxicol Appl Pharmacol* **238**, 90-99.

Larson, J. L., and Bull, R. J. (1992). Species differences in the metabolism of trichloroethylene to the carcinogenic metabolites trichloroacetate and dichloroacetate. *Toxicology and Applied Pharmacology* **115**, 278-285.

Lash, L. H., Lipscomb, J. C., Putt, D. A., and Parker, J. C. (1999a). Glutathione conjugation of trichloroethylene in human liver and kidney: kinetics and individual variation. *Drug Metab Dispos* **27**, 351-359.

Lash, L. H., Parker, J. C., and Scott, C. S. (2000). Modes of action of trichloroethylene for kidney tumorigenesis. *Environ Health Perspect* **108 Suppl 2**.

Lash, L. H., Putt, D. A., Brashear, W. T., Abbas, R., Parker, J. C., and Fisher, J. W. (1999b). Identification of S-(1,2-dichlorovinyl)glutathione in the blood of human volunteers exposed to trichloroethylene. *J Toxicol Environ Health A* **56**, 1-21.

Lash, L. H., Putt, D. A., and Parker, J. C. (2006). Metabolism and tissue distribution of orally administered trichloroethylene in male and female rats: identification of glutathione- and cytochrome P-450-derived metabolites in liver, kidney, blood, and urine. *J Toxicol Environ Health A* **69**, 1285-1309.

Lash, L. H., Qian, W., Putt, D. A., Desai, K., Elfarra, A. A., Sicuri, A. R., and Parker, J. C. (1998). Glutathione conjugation of perchloroethylene in rats and mice in vitro: sex-, species-, and tissue-dependent differences. *Toxicol Appl Pharmacol* **150**, 49-57.

Liebler, D. C., and Guengerich, F. P. (1983). Olefin oxidation by cytochrome P-450: evidence for group migration in catalytic intermediates formed with vinylidene chloride and *trans*-1-phenyl-1-butene. *Biochemistry* **22**, 5482-5489.

Liu, M. Y., Poellinger, L., and Walker, C. L. (2003). Up-regulation of hypoxia-inducible factor 2alpha in renal cell carcinoma associated with loss of Tsc-2 tumor suppressor gene. *Cancer Res* **63**, 2675-2680.

Lumsden, A. B., McLean, A., and Lamb, D. (1984). Goblet and Clara cells of human distal airways: evidence for smoking induced changes in their numbers. *Thorax* **39**, 844-849.

MAK (1996). Trichlorethylene. In *Occupational Toxicants - Critical data evaluation for MAK values and classification of carcinogens by the commission for the investigation of health hazards of chemical compounds in the work area* (H. Greim, Ed.), pp. 201-244. Wiley-VCH, München.

Mally, A., Walker, C. L., Everitt, J. I., Dekant, W., and Vamvakas, S. (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro. *Toxicology* **224**, 108-118.

Miller, R. E., and Guengerich, F. P. (1982). Oxidation of trichloroethylene by liver microsomal cytochrome P-450: evidence for chlorine migration in a transition state not involving trichloroethylene oxide. *Biochemistry* **21**, 1090-1097.

Nickerson, M. L., Jaeger, E., Shi, Y., Durocher, J. A., Mahurkar, S., Zaridze, D., Matveev, V., Janout, V., Kollarova, H., Bencko, V., Navratilova, M., Szeszenia-Dabrowska, N., Mates, D., Mukeria, A., Holcatova, I., Schmidt, L. S., Toro, J. R., Karami, S., Hung, R., Gerard, G. F., Linehan, W. M., Merino, M., Zbar, B., Boffetta, P., Brennan, P., Rothman, N., Chow, W. H., Waldman, F. M., and Moore, L. E. (2008). Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. *Clin Cancer Res* **14**, 4726-4734.

Odum, J., Foster, J. R., and Green, T. (1992). A mechanism for the development of clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chem. Biol. Interact.* **83**, 135-153.

Phimister, A. J., Lee, M. G., Morin, D., Buckpitt, A. R., and Plopper, C. G. (2004). Glutathione depletion is a major determinant of inhaled naphthalene respiratory toxicity and naphthalene metabolism in mice. *Toxicol Sci* **82**, 268-278.

Plopper, C. G., Mariassy, A. T., and Hill, L. H. (1980). Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: I. A comparison of rabbit, guinea pig, rat, hamster, and mouse. *Exp Lung Res* **1**, 139-154.

Plopper, C. G., Van Winkle, L. S., Fanucchi, M. V., Malburg, S. R., Nishio, S. J., Chang, A., and Buckpitt, A. R. (2001). Early events in naphthalene-induced acute Clara cell toxicity. II. Comparison of glutathione depletion and histopathology by airway location. *Am J Respir Cell Mol Biol* **24**, 272-281.

Reed, D. J., Babson, J. R., Beatty, P. W., Brodie, A. E., Ellis, W. W., and Potter, D. W. (1980). High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thiols and disulfides. *Anal Biochem* **106**, 55-62.

Robbiano, L., Baroni, D., Carrozzino, R., Mereto, E., and Brambilla, G. (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney. *Toxicology* **204**, 187-195.

Shultz, M. A., Choudary, P. V., and Buckpitt, A. R. (1999). Role of murine cytochrome P-450 2F2 in metabolic activation of naphthalene and metabolism of other xenobiotics. *J Pharmacol Exp Ther* **290**, 281-288.

Shultz, M. A., Morin, D., Chang, A. M., and Buckpitt, A. (2001). Metabolic capabilities of CYP2F2 with various pulmonary toxicants and its relative abundance in mouse lung subcompartments. *J Pharmacol Exp Ther* **296**, 510-519.

Sweeney, L. M., Kirman, C. R., Gargas, M. L., and Dugard, P. H. (2009). Contribution of trichloroacetic acid to liver tumors observed in perchloroethylene (perc)-exposed mice. *Toxicology* **260**, 77-83.

Swenberg, J. A., and Lehman-McKeeman, L. D. (1999). a2u-Globulin associated nephropathy as a mechanism of renal tubular cell carcinogenesis in male rats. In *IARC-Scientific Publications: Species differencies in thyroid, kidney and urinary bladder carcinogenesis* (C. C. Capen, E. Dybing, J. M. Rice, and J. D. Wilbourn, Eds.), pp. 95-118. International Agency on Cancer Research, Lyon.

Templin, M. V., Parker, J. C., and Bull, R. J. (1993). Relative formation of dichloroacetate and trichloroacetate from trichloroethylene in male B6C3F1 mice. *Toxicology and Applied Pharmacology* **123**, 1-8.

Terracini, B., and Parker, V. H. (1965). A Pathological Study on the Toxicity of S-Dichlorovinyl-L-Cysteine. *Food Cosmet Toxicol* **3**, 67-74.

Toyokuni, S., Okada, K., Kondo, S., Nishioka, H., Tanaka, T., Nishiyama, Y., Hino, O., and Hiai, H. (1998). Development of high-grade renal cell carcinomas in rats independently of somatic mutations in the Tsc2 and VHL tumor suppressor genes. *Jpn J Cancer Res* **89**, 814-820.

Turner, M., Mantick, N. A., and Carlson, G. P. (2005). Comparison of the depletion of glutathione in mouse liver and lung following administration of styrene and its metabolites styrene oxide and 4-vinylphenol. *Toxicology* **206**, 383-388.

Villaschi, S., Giovanetti, A., Lombardi, C. C., Nicolai, G., Garbati, M., and Andreozzi, U. (1991). Damage and repair of mouse bronchial epithelium following acute inhalation of trichloroethylene. *Exp Lung Res* **17**, 601-614.

Völkel, W., Friedewald, M., Lederer, E., Pähler, A., Parker, J., and Dekant, W. (1998). Biotransformation of perchloroethene: dose-dependent excretion of trichloroacetic acid, dichloroacetic acid and *N*-acetyl-*S*-(trichlorovinyl)-L-cysteine in rats and humans after inhalation. *Toxicology and Applied Pharmacology* **153**, 20-27.

West, J. A., Buckpitt, A. R., and Plopper, C. G. (2000a). Elevated airway GSH resynthesis confers protection to Clara cells from naphthalene injury in mice made tolerant by repeated exposures. *J Pharmacol Exp Ther* **294**, 516-523.

West, J. A., Chichester, C. H., Buckpitt, A. R., Tyler, N. K., Brennan, P., Helton, C., and Plopper, C. G. (2000b). Heterogeneity of clara cell glutathione. A possible basis for differences in cellular responses to pulmonary cytotoxicants. *Am J Respir Cell Mol Biol* **23**, 27-36.

West, J. A., Williams, K. J., Toskala, E., Nishio, S. J., Fleschner, C. A., Forman, H. J., Buckpitt, A. R., and Plopper, C. G. (2002). Induction of tolerance to naphthalene in Clara cells is dependent on a stable phenotypic adaptation favoring maintenance of the glutathione pool. *Am J Pathol* **160**, 1115-1127.

Witschi, H. (1991). Lung tumor susceptibility in mice: an overview. *Exp Lung Res* **17**, 281-282.

# APPENDIX 5

## Peer Reviewer Comments on Draft TCE Work Plan Assessment[1]

It is clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan assessment that EPA carried out for TCE. There can be no doubt that this is the proper characterization of the June 2014 assessment. The Chairperson of EPA's peer review panel wrote:

> "The draft document fails to articulate satisfactorily that the analysis described within should be characterized as a screening level assessment. . . . I believe that the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . .
>
> "After listening carefully to the comments and contributions from the other members of the Panel, I have concluded that there would little benefit in revising this draft screening assessment. Rather, I would suggest that the effort be put into a higher tier, more refined assessment which would include empirical data gathered during the course of real-world uses, e.g., as OPP regularly asks be done for occupational exposures and sometimes for residential exposures, consumer use survey data, evaluation of exposure using additional modeling tools and a revisiting and reanalysis of the choices of toxicity and epidemiologic studies used to describe the health benchmark at the MEC99 level and the rationale for selecting the singular MOE of 30 to apply to the selected studies, each of which have varying degrees of credibility. This current draft screening level assessment could then be attached as an appendix to the new second-generation assessment, and described, in summary form, in the early chapter(s) of the new assessment. I would have saved the resources expended for the current external peer review and spent them on the next-generation assessment."
>
> She further stated:
>
> "By selecting the HEC99 and very conservative assumptions about exposure, one ends up with a very conservative (that is, health-protective) risk assessment, which assures only the certainty that the potential risk has not been under-estimated. It does little to resolve the uncertainty of the true estimate of risk."

The Chairperson's main point was that the information (*i.e.*, the screening level assessment) is not consistent with any intended use to support regulation. Her advice was that there would be little benefit in even revising the assessment, given its inadequacy for regulatory use. Taken together, these comments by the Chairperson of EPA's peer review panel establish quite clearly that the TCE risk evaluation does not meet the requirements of new TSCA § 26(h).

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[1] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

One of the peer review panelists, Calvin Willhite, raised serious concerns over the derivation of the non-cancer dose-response:

> "The non-cancer hazard index not only leads to calculation of the lowest equivalent 'safe' concentration of TCE in residential air, but those values are either less than or consistent with background TCE concentrations in United States urban or residential indoor air. As such, any domestic use of TCE in any amount for any use whatsoever will exceed the US EPA's published residential indoor air TCE level (0.21 μg/m3). As written, the previously published and current US EPA reports lead to the conclusion that current ambient TCE levels are associated with increased risk for human cardiovascular malformations - yet there are no suggestions from studies of occupational TCE exposures at concentrations 1-2 magnitude of orders greater than ambient pose excess non-cancer health risks to those workers."

With regard to uncertainty, weight of scientific evidence, quality and reproducibility, and other criteria identified in § 26(h), Dr. Willhite stated:

> "Question 5-4. Please comment on whether the document has adequately described the uncertainties and data limitations. Please comment on whether this information is presented in a transparent manner.
>
> "The general comments concerning the OPPT and IRIS conclusions on risk for cardiovascular malformations above illustrate the poor weight of evidence assessment carried out in this regard for TCE. The uncertainty attendant to the IRIS hazard identification for cardiovascular terata is so great that it leads to the present OPPT conclusion that all TCE exposures (including background concentrations in US urban ambient and indoor residential air) present increased risk for congenital malformation of the heart and great vessels.
>
> "It is not clear why OPPT relied on the results of the Johnson et al. (2003) study to the exclusion of all other inhalation and oral developmental toxicity studies in rodents and rabbits. If in fact the OPPT is reliant upon only the inhalation data, why is it the Carney et al. (2001), the Schwetz et al. (1975), the Hardin et al. (1981), the Beliles et al. (1980) or the Dorfmueller et al. (1979) study was not used? Why is there no discussion of all of the available developmental toxicity inhalation bioassays in the present analysis?
>
> "Summary
>
> "As submitted, the exposure parameters appear arbitrary (e.g., 0.5 and 1 hr/day) and may have been selected for sake of convenience. The data upon which conclusions put forward by OPPT on risk for developmental toxicity associated with arts and crafts use of TCE are not reliable. Nearly all developmental toxicity studies with TCE in rodents find no sign of teratogenicity (e.g., Beliles et al., 1980) or find only slight developmental delay (Dormueller et al., 1979). Chiu et al. (2013) cite the NRC (2006) report as verification of their risk assessment for TCE developmental toxicity, but actually the NRC (2006) concluded:

> 'Additional studies evaluating the lowest-observed-adverse- effect-level and mode of action for TCE-induced developmental effects are needed to determine the most appropriate species for human modeling.'

"In its present assessment, the OPPT ignored the serious deficiencies already identified in conduct of the Johnson et al. (2003) rat drinking water study upon which the BMD01 was based (Kimmel et al., 2009; Watson et al., 2006) [Attachments 1 and 2]. In their weight-of-evidence assessment, Watson et al. (2006) concluded:

> '...application of Hill's causality guidelines to the collective body of data revealed no indication of a causal link between gestational TCE exposure at environmentally relevant concentrations and congenital heart defects.'

"Those conclusions were consistent with Hardin et al. (2005). Perhaps most disturbing of all in US EPA's reliance upon Johnson et al. (2003) as the key study (which for the basis for their lowest non-cancer TCE hazard index and margin of exposure) is the observation by Hardin and associates (2004):

'Conventional developmental and reproductive toxicology assays in mice, rats and rabbits consistently fail to find adverse effects of TCE on fertility or embryonic development aside from embryo- or fetotoxicity associated with maternal toxicity. Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a 'specific' cardiac teratogen.'

"One of the fundamental tenants in science is the reliability and reproducibility of results of scientific investigations. In this regard, one of the most damning of the TCE developmental toxicity studies in rats is that by Fisher et al. (2005) who stated:

'The objective of this study was to orally treat pregnant CDR(CD) Sprague-Dawley rats with large bolus doses of either TCE (500 mg/kg), TCA (300 mg/kg) or DCA (300 mg/kg) once per day on days 6 through 15 of gestation to determine the effectiveness of these materials to induce cardiac defects in the fetus. All-trans-retinoic acid (RA) dissolved in soybean oil was used as a positive control.

"The heart malformation incidence for fetuses in the TCE-, TCA- and DCA-treated dams did not differ from control values on a per fetus or per litter basis. The RA treatment group was significantly higher with 33% of the fetuses displaying heart defects.'

"Unfortunately, Johnson et al. (2005) failed to report the source or age of their animals, their husbandry or provide comprehensive historical control data for spontaneous cardiovascular malformations in their colony. The Johnson study with 55 control litters compared to 4 affected litters of 9 treated was apparently conducted over a prolonged period of time (perhaps years); it is possible this was due to the time required to dissect and inspect fresh rodent fetuses by a small

academic research group. However, rodent background rates for malformations, anomalies and variants show temporal fluctuations (WHO, 1984) and it is not clear whether the changes reported by Johnson et al. (2005) were due to those fluctuations or to other factors. Surveys of spontaneous rates of terata in rats and other laboratory animals are common particularly in pharmaceutical and contract laboratory safety assessment (e.g., Fritz et al., 1978; Grauwiler, 1969; Palmer, 1972; Perraud, 1976). The World Health Organization (1984) advised:

> 'Control values should be collected and permanently recorded. They provide qualitative assurance of the nature of spontaneous malformations that occur in control populations. Such records also monitor the ability of the investigator to detect various subtle structural changes that occur in a variety of organ systems.'

"Rates of spontaneous congenital defects in rodents can vary with temperature and housing conditions. For example, depending on the laboratory levocardia and cardiac hypertrophy occur in rats at background rates between 0.8-1.25% (Perraud, 1976). Laboratory conditions can also influence study outcome; for instance, maternal hyperthermia (as a result of ambient elevated temperature or infection) can induce congenital defects (including cardiovascular malformations) in rodents and it acts synergistically with other agents (Aoyama et al., 2002; Edwards, 1986; Zinskin and Morrissey, 2011). Thus while the anatomical observations made by Johnson et al. (2003) may be accurate, in the absence of data on maternal well-being (including body weight gain), study details (including investigator blind evaluations), laboratory conditions, positive controls and historical rates of cardiac terata in the colony it is not possible to discern the reason(s) for the unconventional protocol, the odd dose-response and marked differences between the Johnson et al. (2003) results and those of other groups.

"As noted by previous investigators, the rat fetus is "clearly at risk both to parent TCE and its TCA metabolite" given sufficiently high prenatal TCE exposures that can induce neurobehavioral deficits (Fisher et al., 1999; Taylor et al., 1985), but to focus on cardiac terata limited to studies in one laboratory that have not been reproduced in other (higher dose) studies and apply the BMD01 with additional default toxicodynamic uncertainty factors appears misleading."

Finally, Michael Jayjock, another peer review panelist, concluded: "Clearly, more work is needed on both the exposure and hazard side of this evaluation to tighten up the exposure assessment and to provide further justification or explanation of the exceedingly low HEC99 values used in the MOE analysis."

As discussed above, other panelists raised serious concerns going to the heart of the "best available science" criteria in TSCA § 26(h). Peer review and public comments identified numerous scientific deficiencies with the draft TCE assessment, including the inappropriate use of default assumptions; ignoring contrary evidence that affects the weight of the scientific evidence; reliance on inapposite exposure data; conclusions inconsistent with the evidence cited;

and, most importantly, reliance on a study that is not reproducible. Equally important deficiencies in both the hazard and exposure assessments were noted.

EPA completely disregarded the peer reviewers' advice and issued the final Work Plan assessment in June 2014 without making any substantial change to the draft. Under TSCA § 26(h), however, EPA must make its science-based decisions "in a manner consistent with the best available science" and "based on the weight of the scientific evidence." In addition, EPA can no longer afford to ignore the conclusions of the peer review it initiated, as it must consider "the extent of independent verification or peer review of the information."